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Subject: Comments on the ACC's test plan for ZDDPs

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cc:

Subject: Comments on the ACC's test plan for ZDDPs

Attached please find the comments of the American animal protection community on the ACC's HPV test plan for ZDDP's.

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HPV test plan comments -- ZDDP.pdf

201-14368

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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March 25, 2003

Christine Todd Whitman, Administrator
US Environmental Protection Agency
Ariel Rios Bldg. (1101A)
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on the HPV test plan for zinc dialkyldithiophosphates

Dear Administrator Whitman:

The following are comments on the test plan for the category zinc dialkyldithiophosphates (ZDDPs), prepared by the Health, Environmental and Regulatory Task Group (HERTG) of the American Chemistry Council's Petroleum Additives Panel. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal, and environmental protection organizations have a combined membership of more than ten million Americans.

Firstly, we would like to commend the test plan in two respects:

(a) *It recognizes the importance of limiting animal testing.*

The test plan includes the following statement:

> In addition to the arguments outline above, HERTG believes that additional testing of zinc dialltyldithiophosphates will cause unnecessary distress to experimental animals. (p. 30)

(b) *It uses chemical categories.*

HERTG has included 12 compounds (CAS nos. 84605-29-8, 68457-79-4, 78784-31-6, 113706-15-3, 26566-95-0, 68988-46-5, 2215-35-2, 4259-15-8, 28629-66-5, 25103-54-2, 54261-67-5 and 11059-65-7) within a single category, termed ZDDPs. Data are thus only required for the category as a whole, or in some cases for a small number of subcategories. The test plan presents a detailed and logical justification for categorization (pp. iii-iv, 1-3), on the basis of the following types of similarity between the nine compounds: (i) molecular structure (each compound consists of a pair of phosphorodithioic acid molecules with two alkyl or alltaryl substituents each, coinplexed with a zinc atom; test plan, pp. 32-35); (ii) physicochemical properties (viscosity, boiling point, vapor pressure and aqueous solubility; test plan, **p.** 36); and (iii) transport, biodegradation and toxicity (as far as is known, they are similar in these respects; test plan, pp. iv-v). In preparing this categorization, HERTG has followed the six steps advocated by the EPA (test plan, p. 3).

HERTG has appropriately decided that no further mammalian tests are needed. However, it has included in the test plan an acute fish toxicity test (OECD guideline 203), to be carried out with three compounds: CAS nos. 84605-29-8, 4259-15-8 and 1159-65-7 **(p.** 16). These tests will kill at least 180 fish.

The first point to make about the plan to carry out fish tests is that it is premature, as the octanol/water partition coefficients of the ZDDPs are not known, and the EPA states that fish tests are only appropriate if the log $K_{o/w}$ value is less than 4.2 (EPA 2000, p. 81695). Tentative data for one ZDDP generated a log $K_{o/w}$ value of 2.49 (test plan, p. 6). However, even if these data are valid, they are for a ZDDP with a relatively small alkyl moiety, so it cannot be assumed that all three of the ZDDPs to be tested have log $K_{o/w}$ values below 4.2. The immediate task now is therefore to determine the log $K_{o/w}$ values of all ZDDPs, or at least the three to be tested, in order to eliminate the testing of those with log $K_{o/w}$ values of 4.2 or higher.

There are several additional reasons why the proposed fish toxicity tests are not appropriate:

(a) ***In vitro*** **and in** ***silico*** **test methods are available**

If HERTG wishes to investigate acute fish toxicity, we urge it to use one or more of the several available *in vitro* and *in silico* methods.

With respect to *in silico* methods, several quantitative structure activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

With respect to *in vitro* methods, TETRATOX, an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997), is the most appropriate. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this pi-esents an ideal opportunity for action rather than words.

The recently validated *Dar*T test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish *(Danio rerio)* eggs as a surrogate for living fish. As the eggs do not hatch during the test period, the test is

classed as a non-animal test. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency; predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1096). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

(b) Previous fish studies have shown an extraordinarily wide range of toxicity

A considerable number of fish studies have been carried out previously. However, as no references are given and little detail is provided, it is not possible to discuss the previous tests with any thoroughness. Notwithstanding this caveat, the fish tests gave LL_{50} and/or EL_{50} values that ranged from less than 0.1 mg/L to more than 100 mg/L (test plan, p. 15). It is possible that this difference was due to physical fouling, in which case chemical toxicity is irrelevant in real-world terms (as discussed below). On the other hand, it is possible that the differences were due to factors other than fouling. However, the test plan offers few suggestions as to what such other factors may have been, other than that the unexpectedly high toxicity may have been due to impurities, or to dissolution above the normal solubility limit (using a co-solvent; p. 15). Whatever the cause of the variability, the test plan inspires no confidence that the tests to be carried out will generate fish toxicity data that are more definitive than were the previous tests.

It is possible that the variation in toxicity was due to the fact that fish of three different genera were used (test plan, p. 38) but it is impossible to discuss this in detail, because the test plan provides no details about individual tests. Indeed, the names of the fish species appear to have been included by mistake, as they are in the footnote to Table 5, but have no connection with the table contents. However, if there really is a high level of interspecies variability in fish toxicity, fish toxicity tests will have virtually no value for predicting the effects of pollution on environmental fish populations.

(c) The ecoiogic relevance of fish toxicity should be taken into consideration

The difference between the purposes of the ecotoxicity and mammalian toxicity tests must be noted. The principle of the mammalian toxicity tests is that they are assumed to be useful for predicting toxicity in individual humans. Fish tests, on the other hand, are not for predicting toxicity in individual fish, but for predicting economic loss (to cominercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The test therefore aims to show whether pollution with ZDDPs will result in large-scale

fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. However, the toxicity of ZDDPs towards these types of organism is unknown, as shown by the inclusion in the test plan of tests on an aquatic crustacean *(Daphnia magna)* and a unicellular green alga (*Pseudokirchneriella szibcapitata).* Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

Physical fouling (discussed below) is important in the context of the food chain, as it tends to have particularly severe effects on phytoplankton, which directly or indirectly support most fish species (Hewstone 1994), and these effects could affect the need for an *in vivo* fish test.

(d) ZDDP exposure causes physical fouling

Viscous, hydrophobic coinpounds such as ZDDPs tend to physically foul aquatic organisms. The test plan suggests that in previous aquatic toxicity tests the measured toxicity was too high, due to physical fouling (coating of fish gills, trapping of invertebrates, etc.). There were also problems such as the formation of oil droplets, a surface sheen (which results in oxygen deficiency in the water), and colloids (p. 15). The formation of a surface sheen is particularly likely in the case of ZDDPs of lower specific gravity than water, and, of a total of 12, the specific gravity of one is lower than water and those of five are unknown (test plan, p. 36).

Associated compounds are also likely to cause fouling. In the case of exposure due to a spill at the manufacturing site, the reaction mixture will contain coinpounds that cause fouling. For example, CAS numbers 64742-54-7 and 64741-88-4, the lubricating oils most commonly added to the ZDDP reaction mixture (test plan, p. 4), are viscous, hydrophobic, and less dense than water (CONCAWE 1997). CAS no. 64941-88-4, in particular, is a component of products known to foul aquatic organisms (Shell 1999). In the case of exposure due to spillage or dumping of new or used commercial products, on the other hand, ZDDPs make up only 0.1-20% of the whole mixture, with the rest being mainly lubricating oil (test plan, p. 6), which causes severe fouling (CONCAWE 1997).

The test plan presents the physical effects merely as interferences with the measurement of chemical toxicity, and thus as difficulties that will have to be overcoine when the fish tests are carried out (p. 15). However, this perspective betrays a concern with abstract chemistry rather than real-world risks. It is likely that physical fouling has more impact on fish survival than chemical toxicity, especially as some measurements of fish toxicity have given LL_{50} values lower than 0.1 mg/L (test plan, p. 15). The test plan itself states that previous LL_{50} values differed by more than three orders of magnitude, and suggested that this may have been due to physical effects (p. 15). It is therefore difficult to see the point of additional toxicity tests: the chemical toxicity of ZDDPs is purely academic if they are responsible for severe physical fouling and always occur in association with other compounds that are responsible for physical fouling.

> We must emphasize that the possibility that fouling is inore important than toxicity does not mean that fish fouling tests should be carried out instead of fish toxicity tests. The severity of fouling can be readily predicted from physical parameters such as specific gravity, hydrophobicity and viscosity. In addition, the ecologic effects of oil spills have been intensively studied. However, rather than additional toxicity data, the high-priority requirement for reducing damage to real-world fish populations is reduction of pollution by, for example, inore rigorous enforcement of legislation against the dumping of used lubricants (Hewstone 1994).

Given the inforination presented above, and the fact that reducing the risk of damage to real-world fish populations is inore important than obtaining abstract data about fish toxicity in laboratories, it is worth reiterating two provisions of the October 1999 agreement to reduce the number of animals killed in the HPV Program, as follows:

(1) In aiialyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that *certain endpoints need not be tested*

(8) ... As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be *useful or relevant*.

Thank you for your attention to these comments. I can be reached at 757-622-7382, extension 1304, or via e-mail at JessicaS@PETA.org.

Yours sincerely,

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References

CONCAWE. 1997. Lubricating oil basestocks. Website: http://www.CONCAWE.be/Download/Reports/Rpt_97-108.pdf.

EPA. 2000. Data collection and development on high production volume (HPV) chemicals. *Federal Register* 65 (248).

EPA. 2002. Ecological structure activity relationships. Website: http://www.epa.gov/opptnewchems 21ecosar.htm.

Friccius T *et al.* 1995. Der Embryotest mit dem Zebrabarbling: Eine Neue Mögligkeit zur Prüfung und Bewertung der Toxizitat von Abwasserproben. *Vom Wasser* 84: 407-418.

Larsen J *et al.* 1997. Progress in an ecotoxicological standard protocol with protozoa: Results from a pilot ring test with *Tetrahymena pyriformis*. *Chemosphere* 35: 1023-41.

Newstone RK. 1994. Health, safety and environmental aspects of used crankcase lubricating oils. *Sci. Total Env.* 156: 255-268.

Nagel R. 1998. *Umweltchemikalien und Fische: Beiträge zu Einer Bewertung,* Johannes Gutenberg Universität, Mainz.

Schulte C *et al.* 1994. Testing acute toxicity in the embryo in zebrafish, *Brachydanio rerio,* as an alternative to the acute fish test: Preliminary results. *ATLA* 22: 12-19.

Schulte *C et al.* 1996. Testing acute toxicity in the embryo of zebrafish *(Brachydanio rerio):* An alternative to the acute fish toxicity test", *Proceedings of the 2nd World Congress on Alternatives and Animal Use in the Life Sciences,* Utrecht, Netherlands.

Scliultz TW. 1997. TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint - a surrogate for fish lethality. *Toxicol. Meth.* 7: 289-309.

Shell. 1999. Safety Data Sheet for ENSIS RX (SDS no. SN1OM086).